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13. ABSTRACT *(Maximum 200 words)*

Pseudomonas that uses TNT as a nitrogen source. Biological remediation of pollutants in soils and groundwater is often desirable due to its relative low cost and effectiveness compared to other treatment technologies. Nitroaromatic compounds such as TNT are resistant to biological oxidation due to the electrophilic nature of the nitro groups and their subsequent stabilization of the aromatic ring. In the past twenty-five years numerous studies have researched methods to bioremediate TNT-contaminated soils and waters. These methods include composting, aerobic slurry reactors, in situ stimulation, anaerobic reactors and studies with pure strains of aerobic and anaerobic bacteria and fungi. Most of these studies have shown that in addition to the productive degradation of TNT, many reactions occur in which partially degraded metabolites of TNT condense and become more recalcitrant than the parent molecule. Recent research on pure strains of aerobic bacteria that use TNT as a nitrogen source has shown, unfortunately, that non-productive reactions (reduction) compete with productive (denitration) reactions during growth on TNT as a sole nitrogen source. We have characterized an aerobic bacterium, which was isolated from a TNT-contaminated site in Armenia. This isolate grows in minimal media with TNT (30-120 ppm) as a sole nitrogen source. A greater understanding of the genes, which catalyze the initial steps of productive TNT metabolism may lead to the construction of hybrids that can degrade TNT in a more efficient manner.

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Newcombe, David. 2001 (expected). Biodegradation of TNT. Ph.D., microbiology, molecular biology, and biochemistry, University of Idaho.

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David A. Newcombe and Ronald L. Crawford 2001. Degradation of Energetic Compounds by Fungi. In, *Fungi in Bioremediation*, (Geoffrey Gadd, ed.) Kluwer Press. in press.

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Newcombe, D, A Paszczynski, L Allenbach, R Crawford. Degradation of 2,4,6-trinitrotoluene by the brown-rot basidiomycete *Gloeophyllum trabeum*. Poster presented at the Second International Symposium on Biodegradation of Nitroaromatic Compounds and Explosives in Leesburg, VA.

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***Pseudomonas* that uses TNT as a nitrogen source.** Biological remediation of pollutants in soils and groundwater is often desirable due to its relative low cost and effectiveness compared to other treatment technologies. Nitroaromatic compounds such as TNT are resistant to biological oxidation due to the electrophilic nature of the nitro

groups and their subsequent stabilization of the aromatic ring. In the past twenty-five years numerous studies have researched methods to bioremediate TNT-contaminated soils and waters. These methods include composting, aerobic slurry reactors, *in situ* stimulation, anaerobic reactors and studies with pure strains of aerobic and anaerobic bacteria and fungi. Most of these studies have shown that in addition to the productive degradation of TNT, many reactions occur in which partially degraded metabolites of TNT condense and become more recalcitrant than the parent molecule. Recent research on pure strains of aerobic bacteria that use TNT as a nitrogen source has shown, unfortunately, that non-productive reactions (reduction) compete with productive (denitration) reactions during growth on TNT as a sole nitrogen source. We have characterized an aerobic bacterium, which was isolated from a TNT-contaminated site in Armenia. This isolate grows in minimal media with TNT (30-120 ppm) as a sole nitrogen source. A greater understanding of the genes, which catalyze the initial steps of productive TNT metabolism may lead to the construction of hybrids that can degrade TNT in a more efficient manner.

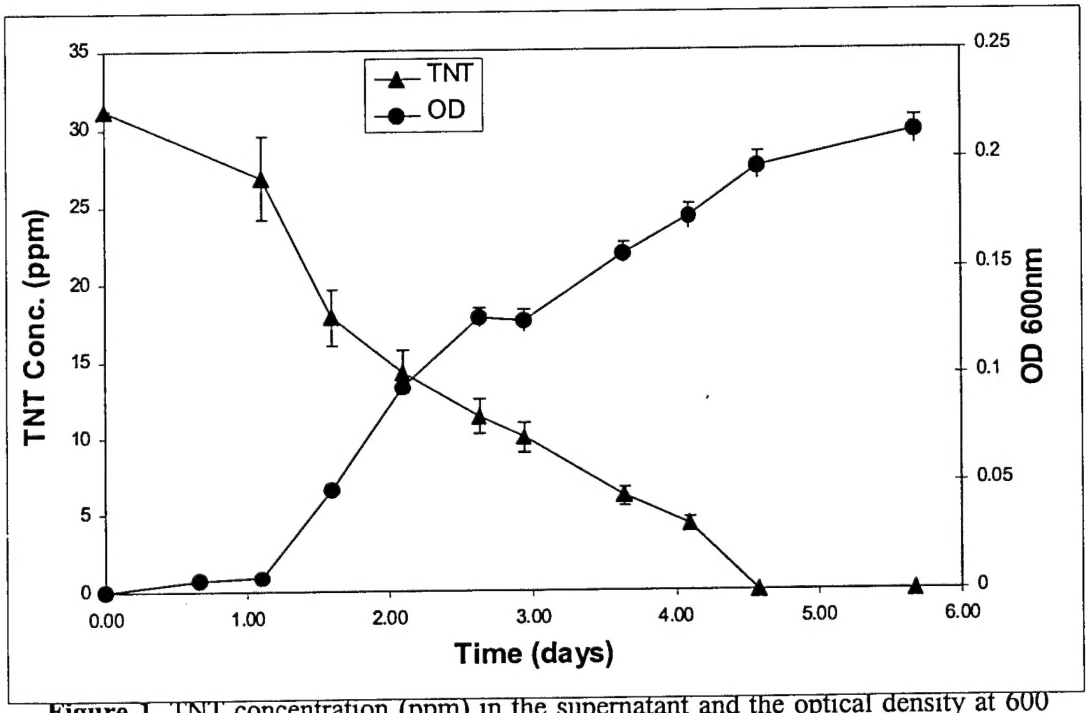

Figure 1. TNT concentration (ppm) in the supernatant and the optical density at 600 nm of a bacterial culture growing in a mineral salts medium with TNT as the sole nitrogen source.

TNT degradation by the brown-rot fungus, *Gloeophyllum trabeum*. During this project we studied the ability of a brown rot fungus, *Gloeophyllum trabeum*, to transform TNT. Initially, we observed that two-week old cultures were able to rapidly transform 50 ppm TNT to below detection limits within 3 days. Metabolites that we have identified at this time by high performance liquid chromatography (HPLC) and gas chromatography/mass spectrophotometery (GC/MS) are 2- and 4-amino-4,6-dinitrotoluene and 2,4-amino-6-nitrotoluene. *G. trabeum* could grow in a minimal salts, nitrogen limiting medium with TNT concentrations ≤40 mg/L These data were

encouraging considering the relative low toxicity threshold of other TNT-degrading wood-rotting fungi such as the white-rot fungus *P. chrysosporium* when growing in the presence of TNT. Work was thus continued on characterization of the degradation pathway and system that may be responsible for these abilities of the fungus to degrade TNT.

Our recent discovery of dimethoxyquinones in liquid cultures of the brown rot fungus *Gloeophyllum trabeum* prompted further investigations into their possible roles as redox cycling molecules in Fenton-like reactions that may represent a primary organic molecule degradation mechanism of brown rot fungi. Thus, we compared various *Gloeophyllum* sp. as to their abilities to produce methoxyquinones, especially 2,5- dimethoxyhydroquinone. Six of eight strains examined produced this compound at maximum concentration ranges from 1 ppm for *G. protractum* to 30 ppm for *G. subferrugineum* and *G. trabeum.* The compound salicylic acid was used as a probe to trap Fenton process-produced hydroxyl radicals in cultures of several of the investigated species. All of the cultures examined degraded salicylic acid readily. Low concentrations of 2,3- and 2,5-dihydroxy benzoic acid were observed by using GC-MS to analyze media from cultures receiving salicylic acid. These products are indicative of the presence of hydroxyl radicals, and are expected from the reactions of hydroxyl radicals with salicylic acid. We also examined the degradation of 2,4,6-trinitrotoluene (TNT) by *G. trabeum*. The fungus transformed TNT rapidly, but ^{14}C-labeled TNT was not converted to $^{14}CO_2$. Mass balance studies indicated that 98% of the radioactivity in added radiolabeled TNT remained in the culture supernatant. Analyses of culture extracts using electrospray MS/MS revealed several aromatic nitro-amines and nitro-aldehydes and their oligomeric coupling products. A Shiff Base reaction mechanism explains the formation of these soluble and less toxic oligomers. The compound 2,2,6,6-tetramethyl-1-piperidynyloxide (TEMPO), a stable free radical, was employed as a trap within *in vitro* reactions containing hydrogen peroxide, 2,5-dimethoxyhydroquinone, and TNT. The coupling products of TEMPO and 2,5-dimethoxyquinone were detected, and are indicative of semi-dimethoxyquinone radical formation. This novel observation implicates the direct involvement of semiquinone radicals in biotransformation reactions observed in fungal cultures containing these compounds. Our observations are further evidence of importance of methoxylated quinones as redox mediators in biodegradation processes carried out by brown-rot fungi.